CLAIMS

1. A method of making a single-stranded nucleic acid molecule having stem and loop formations at the 5′ and 3′ ends thereof, the method comprising:

annealing a first oligonucleotide primer to a sample single-stranded nucleic acid molecule, the first oligonucleotide primer comprising a 3′ end portion which anneals to the sample single-stranded nucleic acid molecule and a 5′ end portion comprising substantially the same nucleotide sequence as an arbitrary region of the sample single-stranded nucleic acid molecule;

extending the first oligonucleotide primer from its 3′ end, using a suitable polymerase, to form a first single-stranded nucleic acid molecule comprising a 5′ end portion comprising a first region and a first complementary region located 5′ terminal which, under suitable conditions, anneal to one another to form a loop;

displacing the first single-stranded nucleic acid molecule from the sample single-stranded nucleic acid molecule;

annealing a second oligonucleotide primer to the first single-stranded nucleic acid molecule, the second oligonucleotide primer comprising a 3′ end portion which anneals to the first single-stranded nucleic acid molecule and a 5′ end portion comprising substantially the same nucleotide sequence as an arbitrary region of the first single-stranded nucleic acid molecule;

extending the second oligonucleotide primer from its 3′ end, using a suitable polymerase, to form a second single-stranded nucleic acid molecule comprising (i) a 3′ end portion complementary to the 5′ end portion of the first single-stranded nucleic acid molecule, the 3′ end portion

comprising the first region located 3′ terminal and the first complementary region which, under suitable circumstances, anneal to one another to form a first loop, and (ii) a 5′ end portion comprising a second complementary region located 5′ terminal and a second region which, under suitable circumstances, anneal to one another to form a second loop; and

displacing the second single-stranded nucleic acid molecule from the first single-stranded nucleic acid molecule, whereby the second single-stranded nucleic acid molecule assumes a conformation with a stem and loop formation formed at both the 3′ end portion and the 5′ end portion.

2. The method according to claim 1, wherein each said extending is carried out using a polymerase having strand displacement activity.

3. The method according to claim 1, wherein said displacing the first single-stranded nucleic acid molecule from the sample single-stranded nucleic acid molecule comprises:

annealing a third oligonucleotide primer to the sample single-stranded nucleic acid molecule at a 3′ side position to where the first oligonucleotide primer anneals thereto; and

extending the third oligonucleotide primer from its 3′ end, using a polymerase having strand displacement activity, to displace the first single-stranded nucleic acid molecule from the sample single-stranded nucleic acid molecule.

4. The method according to claim 1, wherein said displacing the second single-stranded nucleic acid molecule from the first single-stranded nucleic acid molecule comprises:

annealing a fourth oligonucleotide primer to the first single-stranded nucleic acid molecule at a 3′ side position to where the second oligonucleotide primer anneals thereto; and

extending the fourth oligonucleotide primer from its 3′ end, using a polymerase having strand displacement activity, to displace the second single-stranded nucleic acid molecule from the first single-stranded nucleic acid molecule.

5. The method according to claim 1, wherein each said displacing is carried out by heat denaturation.

6. The method according to claims 1, wherein the sample single-stranded nucleic acid molecule is RNA, and said extending the first oligonucleotide primer is conducted by an enzyme having a reverse transcriptase activity.

7. The method according to claim 1 wherein each said extending is carried out in the presence of a melting temperature regulator.

8. The method according to claim 7, wherein the melting temperature regulator is betaine.

9. The method according to claim 8, wherein 0.2 to 3.0 M betaine is present.

10. The method according to claim 1, wherein said displacing the first single-stranded nucleic acid molecule is carried out beginning from where the 3′ end portion of the first oligonucleotide primer annealed to the sample single-stranded nucleic acid molecule and continuing to the 3′ end of the first single-stranded nucleic acid molecule.

11. The method according to claim 1, wherein said displacing the second single-stranded nucleic acid molecule is carried out beginning from where the 3′ end portion of the second oligonucleotide primer annealed to the first single-stranded nucleic acid molecule and continuing to the 3′ end of the second single-stranded nucleic acid molecule.

12. A method of copying a nucleic acid molecule comprising:

A) preparing the second single-stranded nucleic acid molecule according to the method of claim 1, thereby forming a template;

B) extending the 3′ terminal of the template to the 5′ end of the template by means of a polymerase having strand displacement activity, when the first region and first complementary region are annealed to one another to form the first loop, to form a template extension which includes the second complementary region and second region located 3′ terminal, respectively, and which, under suitable conditions, anneal to one another to form a third loop;

C) annealing to the first loop of the extended template an oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least part of the first loop and at the 5′ terminal a nucleotide sequence complementary to the first region of the template; and

D) extending the oligonucleotide primer along the extended template, by means of a polymerase having strand displacement activity, to form a new template complementary to the template formed in step (A).

13. The method according to claim 12 further comprising:

E) displacing the new template from the extended template.

14. The method according to claim 13 wherein said extending in step (D) displaces the template extension formed during said extending in step (B), allowing the second complementary region and the second region to anneal to one another to form a third loop, said displacing in step (E) comprising:

further extending the 3′ terminal of the extended template to the 5′ end of the template, thereby displacing the new template.

15. The method according to claim 14 further comprising:

annealing to the third loop a second oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least a part of the third loop and at the 5′ terminal a nucleotide sequence complementary to the second region of the template; and

extending the 3′ terminal of the second oligonucleotide primer by means of a polymerase having strand displacement activity.

16. The method according to claim 13, further comprising repeating step (B) through (E) using the new template formed in step (D) as the template.

17. The method according to claim 12 wherein each said extending is carried out in the presence of a melting temperature regulator.

18. The method according to claim 17, wherein the melting temperature regulator is betaine.

19. The method according to claim 18, wherein 0.2 to 3.0 M betaine is present.

20. A kit comprising:

a first oligonucleotide primer comprising (i) a 3′ terminal nucleotide sequence that anneals to a sample single-stranded nucleic acid molecule and serves as the origin of synthesis for synthesizing a first single-stranded nucleic acid molecule complementary at least in part to the sample single-stranded nucleic acid molecule, and (ii) a 5′ terminal nucleotide sequence that is complementary to an arbitrary region of the first single-stranded nucleic acid molecule;

a second oligonucleotide primer comprising a nucleotide sequence which anneals to a region of the sample single-stranded nucleic acid molecule located 3′ to where the first oligonucleotide primer anneals thereto;

a third oligonucleotide primer comprising (i) a 3′ terminal nucleotide sequence that anneals to the first single-stranded nucleic acid molecule prepared using the first oligonucleotide primer and serves as the origin of synthesis for synthesizing a second single-stranded nucleic acid molecule complementary at least in part to the first single-

stranded nucleic acid molecule, and (ii) a 5′ terminal nucleotide sequence that is complementary to an arbitrary region of the second single-stranded nucleic acid molecule;

a DNA polymerase having strand displacement activity; and

one or more nucleotides which are used by the DNA polymerase to extend the primers.

21. The kit according to claim 20, further comprising:

a fourth oligonucleotide primer comprising a nucleotide sequence which anneals to a region of the first single-stranded nucleic acid molecule located 3′ to where the third oligonucleotide primer anneals thereto.

22. The kit according to claim 20 further comprising:

a detector for detection of a product of nucleic acid synthesis prepared using the remaining components of the kit.

23. A method of making a single-stranded nucleic acid molecule having stem and loop formations at the 5′ and 3′ ends comprising:

providing a first single-stranded nucleic acid molecule comprising a 5′ end portion comprising a first region located 5′ terminal and a first complementary region which, under suitable conditions, anneal to one another to form a loop;

annealing a first oligonucleotide primer to the first single-stranded nucleic acid molecule, the first oligonucleotide primer comprising a 3′ end portion which anneals to the first single-stranded nucleic acid molecule and

a 5′ end portion comprising substantially the same nucleotide sequence as an arbitrary region of the first single-stranded nucleic acid molecule;

extending the first oligonucleotide primer from its 3′ end, using a suitable polymerase, to form a second single-stranded nucleic acid molecule comprising (i) a 3′ end portion complementary to the 5′ end portion of the first single-stranded nucleic acid molecule, the 3′ end portion comprising a first region located 3′ terminal and a first complementary region which, under suitable circumstances, anneal to one another to form a first loop, and (ii) a 5′ end portion comprising a second region located 5′ terminal and a second complementary region which, under suitable circumstances, anneal to one another to form a second loop;

displacing the second single-stranded nucleic acid molecule from the first single-stranded nucleic acid molecule, beginning from where the 3′ end portion of the first oligonucleotide primer annealed to the first single-stranded nucleic acid molecule and continuing to the 3′ end of the second nucleic acid molecule, wherein, upon displacement of the second single-stranded nucleic acid molecule from the first single-stranded nucleic acid molecule, the second single-stranded nucleic acid molecule assumes a conformation having the 3′ end portion forming the first loop and the 5′ end portion forming the second loop.

24. The method according to claim 23 wherein said extending is carried out using a polymerase having strand displacement activity.

25. The method according to claim 23 wherein said annealing and said extending are carried out in the presence of a melting temperature regulator.

26. The method according to claim 25 wherein the melting temperature regulator is betaine.

27. The method according to claim 26, wherein 0.2 to 3.0 M betaine is present.

28. A method of copying a nucleic acid comprising:

A) preparing the second single-stranded nucleic acid molecule according to the method of claim 23, thereby forming a template;

B) extending the 3′ terminal of the template to the 5′ end of the template by means of a polymerase having strand displacement activity, when the first region and first complementary region are annealed to one another to form the first loop, to form a template extension which includes a third region located 3′ terminal and a third complementary region which are substantially the same as the second complementary region and second region, respectively, and which, under suitable conditions, anneal to one another to form a third loop;

C) annealing to the first loop of the extended template an oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least part of the first loop and at the 5′ terminal a nucleotide sequence complementary to the first region of the template; and

D) extending the oligonucleotide primer along the extended template, by means of a polymerase having strand displacement activity, to form a new template complementary to the template formed in step (A).

29. The method according to claim 28 further comprising:

E) displacing the new template from the extended template.

30. The method according to claim 29 wherein said extending in step (D) displaces the template extension formed during said extending in step (B), allowing the third region and the third complementary region to anneal to one another to form a third loop, said displacing in step (E) comprising:

further extending the 3′ terminal of the extended template to the 5′ end of the template, thereby displacing the new template.

31. The method according to claim 30 further comprising:

annealing to the third loop a second oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least a part of the third loop and at the 5′ terminal a nucleotide sequence complementary to the third region of the template; and

extending the 3′ terminal of the second oligonucleotide primer by means of a polymerase having strand displacement activity.

32. The method according to claim 29, wherein the new template has (i) a 5′ end portion comprising the first region and the first complementary region located 5′ terminal which, under suitable conditions, anneal to one another to form the first loop, and (ii) a 3′ end portion comprising the second region and the second complementary region located 3′ terminal which, under suitable conditions, anneal to one another to form the second loop, said method further comprising:

F) extending the 3′ terminal of the new template to the 5′ end of the new template by means of a polymerase having strand displacement activity, when the second region and second complementary region are annealed to one another to form the second loop, to form a template extension which includes a third region and a third complementary region that are substantially the same as the first complementary region and first region, respectively, and which, under suitable conditions, anneal to one another to form a third loop;

G) annealing to the second loop of the extended new template a second oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least a part of the second loop and at the 5′ terminal a nucleotide sequence complementary to the second complementary region of the template;

H) extending the second oligonucleotide primer along the extended new template, by means of a polymerase having strand displacement activity, to form a third template which is substantially the same as the template.

33. The method according to claim 32 further comprising:

I) displacing the third template from the new template.

34. The method according to claim 33 further comprising:

repeating steps (B) through (I) using the third template.

35. The method according to claim 28 wherein each said extending is carried out in the presence of a melting temperature regulator.

36. The method according to claim 35, wherein the melting temperature regulator is betaine.

37. The method according to claim 36, wherein 0.2 to 3.0 M betaine is present.

38. A method of making a single-stranded nucleic acid molecule having stem and loop formations at the 5′ and 3′ ends thereof, the method comprising:

annealing a first oligonucleotide primer to a sample single-stranded nucleic acid molecule, the first oligonucleotide primer comprising a 3′ end portion which anneals to the sample single-stranded nucleic acid molecule and a 5′ end portion comprising substantially the same nucleotide sequence as an arbitrary region of the sample single-stranded nucleic acid molecule;

extending the first oligonucleotide primer from its 3′ end, using a suitable polymerase, to form a first single-stranded nucleic acid molecule comprising a 5′ end portion comprising a first region and a first complementary region located 5′ terminal which, under suitable conditions, anneal to one another to form a loop;

displacing the first single-stranded nucleic acid molecule from the sample single-stranded nucleic acid molecule, beginning from where the 3′ end portion of the first oligonucleotide primer annealed to the sample single-stranded nucleic acid molecule and continuing to the 3′ end of the first single-stranded nucleic acid molecule;

annealing a second oligonucleotide primer to the first single-stranded nucleic acid molecule, the second oligonucleotide primer comprising a 3′ end portion which anneals to the first single-stranded nucleic acid molecule and a 5′ end portion comprising substantially the same nucleotide sequence as an arbitrary region of the first single-stranded nucleic acid molecule;

extending the second oligonucleotide primer from its 3′ end, using a suitable polymerase, to form a second single-stranded nucleic acid molecule comprising (i) a 3′ end portion complementary to the 5′ end portion of the first single-stranded nucleic acid molecule, the 3′ end portion comprising the first region located 3′ terminal and the first complementary region which, under suitable circumstances, anneal to one another to form a first loop, and (ii) a 5′ end portion comprising a second region located 5′ terminal and a second complementary region which, under suitable circumstances, anneal to one another to form a second loop; and

displacing the second single-stranded nucleic acid molecule from the first single-stranded nucleic acid molecule, whereby the second single-stranded nucleic acid molecule assumes a conformation with a stem and loop formation formed at both the 3′ end portion and the 5′ end portion.

39. The method according to claim 38, wherein each said extending is carried out using a polymerase having strand displacement activity.

40. The method according to claim 38, wherein each said annealing and each said extending is carried out in the presence of a melting temperature regulator.

41. The method according to claim 40, wherein the melting temperature regulator is betaine.

42. The method according to claim 41, wherein 0.2 to 3.0 M betaine is present.

43. The method according to claim 38, wherein said displacing the second single-stranded nucleic acid molecule is carried out beginning from where the 3′ end portion of the second oligonucleotide primer annealed to the first single-stranded nucleic acid molecule and continuing to the 3′ end of the second single-stranded nucleic acid molecule.

44. A method of copying a nucleic acid molecule comprising:

A) preparing the second single-stranded nucleic acid molecule according to the method of claim 38, thereby forming a template;

B) extending the 3′ terminal of the template to the 5′ end of the template by means of a polymerase having strand displacement activity, when the first region and first complementary region are annealed to one another to form the first loop, to form a template extension which includes a third region located 3′ terminal and a third complementary region which are substantially the same as the second complementary region and second region, respectively, and which, under suitable conditions, anneal to one another to form a third loop;

C) annealing to the first loop of the extended template an oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least part of the first loop and at the 5′ terminal a nucleotide sequence complementary to the first region of the template; and

D) extending the oligonucleotide primer along the extended template, by means of a polymerase having strand displacement activity, to form a new template complementary to the template formed in step (A).

45. The method according to claim 44 further comprising:

E) displacing the new template from the extended template.

46. The method according to claim 45 wherein said extending in step (D) displaces the template extension formed during said extending in step (B), allowing the third region and the third complementary region to anneal to one another to form a third loop, said displacing in step (E) comprising:

further extending the 3′ terminal of the extended template to the 5′ end of the template, thereby displacing the new template.

47. The method according to claim 46 further comprising:

annealing to the third loop a second oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least a part of the third loop and at the 5′ terminal a nucleotide sequence complementary to the third region of the template; and

extending the 3′ terminal of the second oligonucleotide primer by means of a polymerase having strand displacement activity.

48. The method according to claim 44, wherein the new template has (i) a 5′ end portion comprising the first region and the first complementary region located 5′ terminal

which, under suitable conditions, anneal to one another to form the first loop, and (ii) a 3′ end portion comprising the second region and the second complementary region located 3′ terminal which, under suitable conditions, anneal to one another to form the second loop, said method further comprising:

F) extending the 3′ terminal of the new template to the 5′ end of the new template by means of a polymerase having strand displacement activity, when the second region and second complementary region are annealed to one another to form the second loop, to form a template extension which includes a third region and a third complementary region that are substantially the same as the first complementary region and first region, respectively, and which, under suitable conditions, anneal to one another to form a third loop;

G) annealing to the second loop of the extended new template a second oligonucleotide primer comprising at the 3′ terminal a nucleotide sequence complementary to at least a part of the second loop and at the 5′ terminal a nucleotide sequence complementary to the second complementary region of the template;

H) extending the second oligonucleotide primer along the extended new template, by means of a polymerase having strand displacement activity, to form a third template which is substantially the same as the template.

49. The method according to claim 48 further comprising:

I) displacing the third template from the new template.

50. The method according to claim 49 further comprising:

repeating steps (B) through (I) using the third template.

51. The method according to claim 44 wherein each said extending is carried out in the presence of a melting temperature regulator.

52. The method according to claim 51, wherein the melting temperature regulator is betaine.

53. The method according to claim 52, wherein 0.2 to 3.0 M betaine is present.